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# Preparation of Black Soybean Peptide and Its Effect on Weight Reduction in Rats

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## ABSTRACT

The large-scale preparation method for black soybean peptide (BSP; about 70% less than 10,000 Da; highly enriched with glutamic acid, aspartic acid, and arginine) was developed, and its effect on weight reduction and lipid profiles in rats was investigated. Sprague-Dawleymale rats were assigned to four dietary groups (high-fat diets containing 0, 2, 6, and 10% BSP) and fed four weeks to examine the effects of BSP. During the experiment, food intake was measured every two days and body weight was monitored two times a week. After the supplementation of BSP, liver and adipose tissues (epididymal, retroperitoneal and perirenal adipose tissue) in the rats were weighted and the lipid profiles in serum, liver, and feces were analyzed. At the results of body weight gain, liver and epididymal adipose tissue weight, BSP groups were more decreased than HF group (0% BSP), with greater decreases at higher BSP levels. The same patterns were shown in lipid profiles of serum, as BSP was increased, triglyceride and total cholesterol concentration decreased. The serum HDL-cholesterol level was increased with increasing at BSP levels. Total cholesterol concentration of liver and feces were decreased and increased, respectively, as BSP increased. The results confirm that BSP is involved in reducing the body weight and the improvement of lipid composition in serum and liver of rats and that BSP can be applied in weight reduction in the food products industry.

**Key words:** black soybean peptide, weight reduction, lipid profiles, triglyceride, cholesterol

## INTRODUCTION

Obesity has been reached the most serious common eating disorder, an imbalance of energy intake and consumption, around the World. Obesity is closely associated with a many of chronic diseases. Therefore, it has taken as a serious public health problem in many countries. The number of obesity in Korea is also increasing yearly due to changes of life style and food intake. Since obesity is known to be related to hyperlipidemia, insulin and leptin resistance, and other chronic diseases, recent research has focused on functional food materials and their anti-obesity activity (Aoyama et al., 2000; Choi et al., 1994).

Soybean protein and/or peptide are one of the most potential agents for applied as functional food material. Recently, various peptides derived from soybean materials were revealed in physiological activities such as angiotensin converting enzyme (ACE) inhibitor, certain anti-cancer, cholesterol reducers, immunomodulators, and so on. Although many soybean peptides have been evaluated in the biological activities, few studies have been reported on soybean peptide for its effect on anti-obesity activity (Lee, 1998).

The large-scale preparation method for black soybean peptide (BSP) was developed. Thus, the objective of this study was to determine the effect of BSP on weight reduction and lipid profiles in rats fed high-fat diet.

## MATERIALS AND METHODS

### Preparation and analysis for black soybean peptide

BSP was produced by enzyme hydrolysis. The preparation process of BSP was shown in Fig. 1. The composition of amino acid and molecular weight profile for BSP were analyzed

Black soybean
↓ Enzyme hydrolysis
Filtering & Concentration
↓ Concentrated, 50 Bx
Black soybean concentrate
↓ 1st protease (2 hrs)
↓ 2nd protease (5 hrs)
Inactivation the enzyme
↓ Concentrated, 30 Bx
Black soybean peptide powder

**Fig. 1.** The preparation process of black soybean peptide.

### Experimental design and diets of animals

Four-wk-old, male, Spraque-Dawley rats, weighing 80~90 g, were obtained from Samtako (Gyeonggi-do, Korea) and fed commercial pellets for 7 days for adaptation. And then they were randomly divided into four groups of eight animals (Table 1). The composition of experimental diets was shown in Table 2. The rats fed AIN-93 diet supplemented (Reeves, 1997) with freely access to the diets and fresh tap water. The diets were similar except for the protein source with or without BSP. They contained 20% (w/w) protein (BSP or casein), 36% (w/w) fat (soybean oil and beef tallow) and 1% (w/w) cholesterol.

The animals were housed individually in stainless steel cages. The temperature (22±2°C) and humidity (65±2%) of the animal room were constant and the rats were kept under a daily inverted light-dark cycle (light: 09:00 to 21:00).

### Food intake, body weight gain and feeding efficiency

Food intake was measured every two days and body weight was monitored two times a week. Feeding efficiency (FER) was calculated by using the formula underneath.

**Table 1.** Classification of experimental groups

| Group[1)] | Food diet |
|---|---|
| HF | High fat diet + cholesterol 1% + casein 20% |
| BSP2 | High fat diet + cholesterol 1% + casein 18% + BSP 2% |
| BSP6 | High fat diet + cholesterol 1% + casein 14% + BSP 6% |
| BSP10 | High fat diet + cholesterol 1% + casein 10% + BSP 10% |

[1)]HF: high fat diet with 0% black soybean peptide.
BSP2: high fat diet with 2% black soybean peptide.
BSP6: high fat diet with 6% black soybean peptide.
BSP10: high fat diet with 10% black soybean peptide.

**Table 2.** The composition of experimental diets (g/100 g diet)

| Ingredients | Experimental diet groups | | | |
|---|---|---|---|---|
| | HF | BSP2 | BSP6 | BSP10 |
| Casein | 20.0 | 18.0 | 14.0 | 10.0 |
| BSP[1)] | - | 2.0 | 6.0 | 10.0 |
| L-Cystine | 0.3 | 0.3 | 0.3 | 0.3 |
| Corn starch | 41.95 | 41.95 | 41.95 | 41.95 |
| Sucrose | 10.0 | 10.0 | 10.0 | 10.0 |
| Cellulose | 5.0 | 5.0 | 5.0 | 5.0 |
| Soybean oil | 7.0 | 7.0 | 7.0 | 7.0 |
| Beef tallow | 10.0 | 10.0 | 10.0 | 10.0 |
| Cholesterol | 1.0 | 1.0 | 1.0 | 1.0 |
| Mineral mix[2)] | 3.5 | 3.5 | 3.5 | 3.5 |
| Vitamin mix[3)] | 1.0 | 1.0 | 1.0 | 1.0 |
| TBHQ (t-Butylhydroquinone) | 0.0014 | 0.0014 | 0.0014 | 0.0014 |
| Choline bitartrate | 0.25 | 0.25 | 0.25 | 0.25 |

[1)]BSP: black soybean peptide.
[2)]Vitamin mixture: AIN-93VX.
[3)]Mineral mixture: AIN-93G.

$$\text{Feeding efficiency (FER, \%)} = \frac{\text{Body weight gain (g/day)}}{\text{Food intake (g/day)}} \times 100$$

**Preparation of serum, liver and adipose tissue samples**

At the end of experiment, animals were deprived of food overnight and sacrificed under dry ice anesthesia. Blood samples were collected from the heart into a heparinized tube. After blood collection, livers and adipose tissue (epididymal, Retroperitroneal, and perirenal adipose tissue) were perfused with ice-cold 0.9% saline solution. Blood samples were centrifuged on at 4,000×g for 15 min to isolate serum and then frozen at -70°C until analyzed. The liver and adipose tissue was harvested, washed with ice-cold isotonic saline, weighted and then stored at -70°C for laboratory analysis. During the 4 weeks feeding period, feces were collected for the last 3 days of the experimental period and then stored at -70°C until analyzed.

**Lipid analysis of serum, liver, and feces**

Serum was used for determination of total cholesterol (TC) and triglyceride (TG) concentrations were measured colorimetrically with commercially available kits (Asan Co., Korea). LDL-C and VLDL-C concentrations were determined by the method of Fridewald et al. (1972), which assumed that circulating VLDL-C consists of 80% triglycerides and 20% cholesterol. Serum concentrations of HDL-C were assayed by the same method, as was serum TC after removal of LDL-C and VLDL-C with magnesium dextran sulfate (Bucolo and David, 1973). Atherogenic index (AI) was calculated according to the method of Haglund et al. (1991). Liver lipids were extracted according to the method of Folch et al. (1957). Briefly, 1 g of liver tissue sliced small pieces and homogenized with chloroform/methanol (2:1, v/v) and then added 2 mL of ice-cold distilled water. After filtration of extracts, the collected solution was centrifuged at 3,000×g for 10 min. The chloroform phase was collected, vaccum-concentrated and then liver TC and TG concentrations were determined by the same method as described in serum with a slight modification by the method of Sale et al. (1984). Feces were dried by dry oven at 80°C for 24 hr and then powdered. The lipid profile in sample was determined by the same method as described in liver.

### Statistical analysis

Results were expressed as means±standard deviation. Comparisons between groups were made using one-way analysis of variance (ANOVA) using SPSS (statistical package for social science) program. Duncan's Multiple Range Test was performed to identify significant differences among groups of rats fed BSP diets. Differences were considered significant when the p value was less than 0.05.

## RESULTS AND DISCUSSION

### Amino acid composition and molecular weight for black soybean peptide

The results of amino acid composition and molecular weight profile of BSP were shown in Table 3 and 4, respectively.

**Table 3.** Amino acid composition of black soybean peptide

| Amino acid | Contents (mg/g) | Amino acid | Contents (mg/g) |
|---|---|---|---|
| Alanine | 1.72 | Lysine | 4.19 |
| Arginine | 10.37 | Methionine | 2.55 |
| Aspartic acid | 13.50 | Phenylalanine | 6.21 |
| Cystine | - | Proline | 6.12 |
| Glutamic acid | 22.36 | Serine | 5.25 |
| Glycine | 4.42 | Threonine | 4.10 |
| Histidine | 3.31 | Tryptophan | |
| Isoleucine | 5.78 | Tyrosine | 4.34 |
| Leucine | 7.73 | Valine | 5.78 |

**Table 4.** Size exclusion HPLC elution profile of peptides isolated from black soybean peptide

| Peptide | Molecular weight distribution (%)[2] | | | | |
|---|---|---|---|---|---|
| | M1 | M2 | M3 | M4 | M5 |
| BSP[1] | 1.9 | 6.4 | 13.1 | 59.6 | 19.0 |

[1]BSP: black soybean peptide.
[2]Degree of molecular weight distribution was expressed as the percentage of each area integration size exclusion HPLC. M1=50 kDa, M2=50～20 kDa, M3=20～10 kDa, M4=10～1 kDa, M5=1 kDa.

### Food intake, body weight gain andfeeding efficiency

Food intake, body weight gain and feeding efficiency (FER) of rats fed the various diets were presented in Table 5 and the change in body weights of the groups during the experimental period was shown in Fig. 2. From Table 5, food intake was no significant difference among the four dietary groups. But the body weight gain and FER in BSP groups were significantly ($p<0.05$) lower than in HF group. Particularly, the body weight gain in BSP10 group was the lowest among in the groups. As BSP level was increased, the body weight gain significantly ($p<0.05$) decreased. The relationship between the content of BSP and weight reduction was demonstrated.

### Analysis of serum lipid profiles

The results of triglyceride, cholesterol, and Atherogenic Index (AI) in serumwere shown in Table 6. It was shown in trends between TG and BSP levels which decreased in TG with increasing BSP levels. Also, there was found in the relationship between cholesterol data and BSP levels. TC and LDL-C were decreased as BSP levels increased. The pattern is opposite to that of HDL-C vs. BSP levels. AI level in BSP groups was significantly

**Table 5.** Food intake, body weight gain and feeding efficiency in rats fed high-fat diet for 4 weeks

| Groups | Food intake (g/day) | Body weight gain (g/day) | FER[1)] |
|---|---|---|---|
| HF | 18.85±1.27[2)NS3)] | 6.80±0.25[d4)] | 0.36±0.01[c] |
| BSP2 | 20.12±1.07 | 6.07±0.22[c] | 0.31±0.02[b] |
| BSP6 | 18.05±0.30 | 5.32±0.62[b] | 0.31±0.03[b] |
| BSP10 | 18.00±2.31 | 3.62±0.81[a] | 0.20±0.03[a] |

[1)]FER: Food efficiency ratio = body weight gain (g/day) / food intake (g/day).
[2)]Values are mean±SD of eight rats in each group.
[3)]NS: not significant.
[4)]Values within the same column with different superscript letters are significantly different from each other at $p<0.05$ by Duncan's multiple range test.

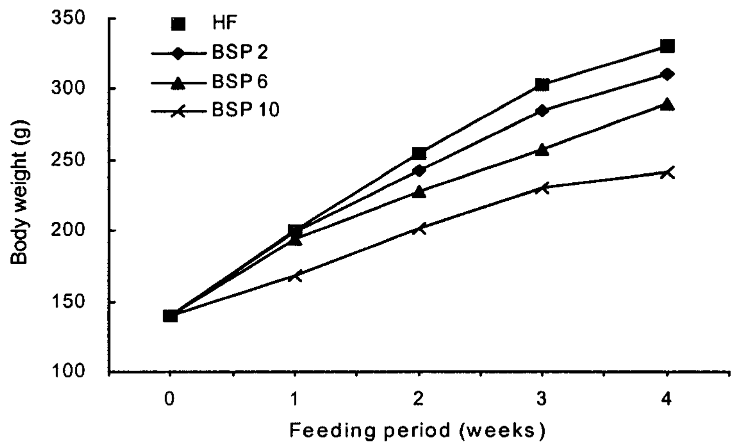


**Fig. 2.** Body weight changes in rats during the experimental period.

**Table 6.** Effect of experimental diets on serumtriglyceride and cholesterollevel in rats fed high-fat diet for 4 weeks

| Groups | Triglyceride (TG) (mg/dL) | Cholesterol (mg/dL) | | | | AI[3)] |
|---|---|---|---|---|---|---|
| | | Total Cholesterol (TC) | HDL cholesterol | LDL cholesterol[1)] | VLDL cholesterol[2)] | |
| HF | 94.28±22.85[4)NS5)] | 126.90±17.72[b6)] | 20.73±3.87[a] | 87.55±18.89[c] | 18.62±4.92[NS] | 5.36±1.80[c] |
| BSP2 | 89.86±18.35 | 118.28±17.41[b] | 26.80±7.85[ab] | 73.51±18.75[bc] | 17.97±3.67 | 3.71±1.62[b] |
| BSP6 | 77.72±33.99 | 109.45±13.50[ab] | 29.23±1.35[b] | 64.68±10.53[b] | 15.54±6.80 | 2.74±0.38[b] |
| BSP10 | 69.58±11.45 | 94.96±14.71[a] | 46.15±6.23[c] | 34.89±11.95[a] | 13.92±2.29 | 1.07±0.30[a] |

[1)]LDL-C = Total cholesterol − (HDL cholesterol + trigylceride/5).
[2)]VLDL-C = Total cholesterol − (HDL cholesterol + LDL-cholesterol).
[3)]Atherogenic index: AI = (Total cholesterol − HDL cholesterol) / HDL cholesterol.
[4)]Values are mean±SD of eight rats in each group.
[5)]NS: not significant.
[6)]Values within the same column with different superscript letters are significantly different from each other at $p<0.05$ by Duncan's multiple range test.

($p<0.05$) lower than in HF group, with greater decreases at higher BSP levels.

**Analysis of liver lipid profiles**

The results of liver lipid profiles were shown in Table 7. There were shown in trends between liver weight/

**Table 7.** Effect of experimental diets on liver total cholesterol, triglyceride total lipid level in rats fed high-fat diet for 4 weeks

| Groups | Liver weight (g) | Total lipid (mg/g liver) | Triglyceride (mg/g liver) | Total cholesterol (mg/g liver) |
|---|---|---|---|---|
| HF | 13.24±1.13[1)c2)] | 557.27±71.05[b] | 106.20±4.95[b] | 140.99±5.37[ab] |
| BSP2 | 12.05±0.39[b] | 511.12±47.27[ab] | 106.63±3.32[b] | 144.50±10.01[b] |
| BSP6 | 10.67±0.22[a] | 469.05±43.20[a] | 109.08±3.36[b] | 133.25±9.03[a] |
| BSP10 | 10.06±0.26[a] | 451.97±71.14[a] | 94.30±11.99[a] | 131.79±8.22[a] |

[1)]Values are mean±SD of eight rats in each group.
[2)]Values within the same column with different superscript letters are significantly different from each other at $p<0.05$ by Duncan's multiple range test.

total lipid and BSP levels which decreased in liver weight (or total lipid) with increasing BSP levels. However, there was no relationship between TG (or TC) and BSP levels. At the point of view in BSP10 group, all liver lipid analyses were significantly ($p<0.05$) decreased compared with HF control group.

**Analysis offecal lipid profiles**

The results of triglyceride, total cholesterol, and total lipid in feceswere shown in Table 8. Although no trend were shown for fecal dry weight, TG, and TC with BSP levels, total lipid analyses with increasing BSP levels showed increasing the numerical number. In fecal total lipid and TC, no significant differences were found among the groups but higher numbers were obtained in BSP groups than in HF control group. Compared with HF group at fecal total lipid, BSP2, 6, and 10 were increased by about 21, 31, and 43%, respectively.

All of the above lipid analysis (serum, liver, feces) data indicate that BSP can improve the lipid composition in rats fed high-fat diet.

**Table 8.** Effect of experimental diets on fecaltotal lipid andtotal cholesterol, triglyceride level in rats fed high-fat diet for 4 weeks

| Groups | Fecal dry weight (g/day) | Total lipid (mg/g feces) | Triglyceride (mg/g feces) | Total cholesterol (mg/g feces) |
|---|---|---|---|---|
| HF | 1.86±0.10[1)b2)] | 203.33±45.09[NS3)] | 7.58±1.47[NS] | 15.48±0.78[NS] |
| BSP2 | 1.15±0.30[a] | 245.00±49.50 | 6.87±0.36 | 17.73±0.35 |
| BSP6 | 1.00±0.00[a] | 265.00±35.36 | 6.34±0.14 | 16.83±0.11 |
| BSP10 | 1.92±0.14[b] | 290.00±17.32 | 7.41±0.49 | 18.00±2.13 |

[1)]Values are mean±SD of eight rats in each group.
[2)]Values within the same column with different superscript letters are significantly different from each other at $p<0.05$ by Duncan's multiple range test.
[3)]NS: not significant.

**Weight of adipose tissue**

Fig. 3 shows the result of the adipose tissue weight such as epididymal, retroperitoneal and erirenal adipose tissue. There were no significant differences of the retroperitoneal and perirenal adipose tissue among the groups, but epididymal adipose tissue weight was decreased with increasing BSP levels.

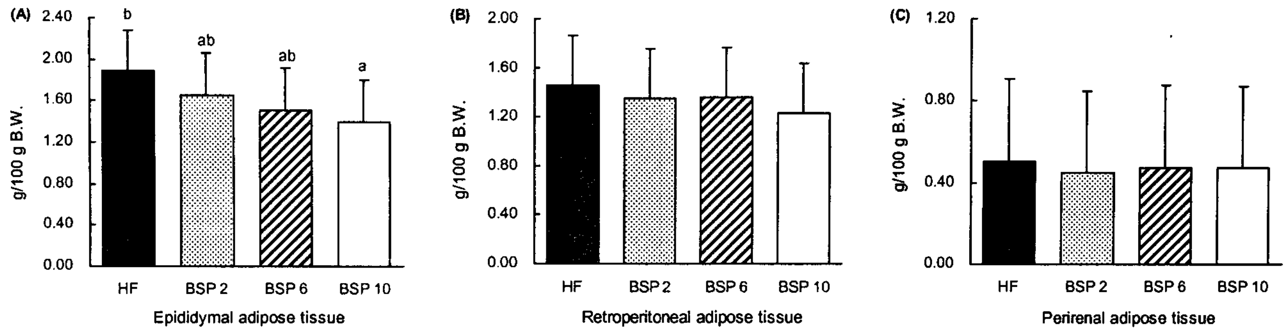


**Fig. 3.** Effect of black soybean peptide on adipose tissue mass.
(A) Epididymal adipose tissue, (B) Retroperitoneal adipose tissue, (C) Perirenal adipose tissue.
Levels of tissue mass were calculated as a weight per 100 g body weight.
Different letters indicate significant difference at $p<0.05$ by Duncan's multiple range test.

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